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Mutagenesis of Bordetella pertussis with transposon Tn5tac1: conditional expression of virulence-associated genes.

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The Tn5tac1 transposon contains a strong outward-facing promoter, Ptac, a lacI repressor gene, and a selectable Kanr gene. Transcription from Ptac is repressed by the lacI protein unless an inducer (isopropyl-beta-D-thiogalactopyranoside [IPTG]) is present. Thus, Tn5tac1 generates insertion mutations in Escherichia coli with conditional phenotypes because it is polar on distal gene expression when IPTG is absent and directs transcription of these genes when the inducer is present. To test the usefulness of Tn5tac1 in Bordetella pertussis, a nonenteric gram-negative bacterial pathogen, we chose the bifunctional adenylate cyclase-hemolysin determinant as an easily scored marker to monitor insertional mutagenesis. Tn5tac1 delivered to B. pertussis on conjugal suicide plasmids resulted in Kanr exconjugants at a frequency of 10(-3) per donor cell, and nonhemolytic (Hly-) mutants were found among the Kanr colonies at a frequency of about 1%. Of eight independent Kanr Hly- mutants, two were conditional and exhibited an Hly+ phenotype only in the presence of IPTG. Using a new quantitative assay for adenylate cyclase based on high-pressure liquid chromatography, we found that enzymatic activity in these two strains was specifically induced at least 500-fold in a dose-dependent fashion over the range of 0 to 125 microM IPTG. These data show that Ptac serves as a promoter, lacI is expressed and is functional, and IPTG can induce Ptac transcription in B. pertussis. Adenylate cyclase expression in whole cells, culture supernatants, and cell extracts from these strains depended upon IPTG, suggesting that the insertions do not merely alter secretion of adenylate cyclase-hemolysin.(ABSTRACT TRUNCATED AT 250 WORDS)